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Ligands of the receptor for advanced glycation end products, including high-mobility group box 1, limit bacterial dissemination during *Escherichia coli* peritonitis

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Abstract

Objective—The receptor for advanced glycation end products mediates a variety of inflammatory responses. Soluble receptor for advanced glycation end products has been suggested to function as a decoy abrogating cellular activation. High-mobility group box 1 is a high-affinity binding ligand for the receptor for advanced glycation end products with cytokine activities and plays a role in sepsis.

Design—Controlled, *in vivo* laboratory study.

Setting—Research laboratory of a health sciences university.

Subjects—C57BL/6 mice.

Interventions—Peritonitis was induced by intraperitoneal injection of *Escherichia coli*. Mice received soluble receptor for advanced glycation end products or anti-high-mobility group box 1 immunoglobulin G, or the appropriate control treatment.

Measurements and Main Results—Soluble receptor for advanced glycation end products-treated mice demonstrated an enhanced bacterial dissemination to liver and lungs, accompanied by increased hepatocellular injury and exaggerated systemic cytokine release, 20 hrs after intraperitoneal administration of *Escherichia coli*. Soluble receptor for advanced glycation end products administration in healthy, uninfected mice did not induce an immune response. Remarkably, lung inflammation was unaffected. Furthermore, high-mobility group box 1 release was enhanced during peritonitis and anti-high-mobility group box 1 treatment was associated with higher bacterial loads in liver and lungs.

Conclusions—These data are the first to suggest that receptor for advanced glycation end products ligands, including high-mobility group box 1, limit bacterial dissemination during Gram-negative sepsis.

Keywords

sepsis; peritonitis; host defense; soluble receptor for advanced glycation end products (sRAGE); high mobility group box (HMGB) 1; *Escherichia coli*

Escherichia coli is the most common pathogen involved in intra-abdominal infection in humans (1). Peritonitis is a life-threatening infection characterized by the presence of bacteria in the normally germ-free peritoneal cavity. Mortality rates of peritonitis range between 30% and 50% despite advances in surgery and antimicrobial therapy. Serious complications originating from peritonitis are systemic inflammation and sepsis, with mortality rates of up to 80% (2).

The multiligand receptor for advanced glycation end products (RAGE) is a member of the immunoglobulin superfamily, being expressed as a cell surface molecule by a range of cell types and playing a key role in diverse inflammatory processes (3). Known RAGE ligands include amyloid (4), β -sheet fibrils (5), and some members of the S100 family, such as S100A12 (6), S100B (7), and S100P (8). Another high-affinity binding ligand for RAGE is high-mobility group box (HMGB)1 (9, 10). HMGB1 is a potent proinflammatory cytokine that plays an important role in a variety of inflammatory conditions, including sepsis and arthritis (11, 12). Engagement of these and other ligands to RAGE can induce inflammatory responses via activation of several intracellular signaling cascades, including the nuclear factor- κ B pathway (13, 14).

Soluble RAGE (sRAGE), a truncated form of the full-length cell surface receptor, is composed of the extracellular ligand-binding domain (V-C-C') only, lacking the cytosolic and transmembrane domains (i.e., the parts that transfer a signal into the cell). sRAGE has been suggested to be involved in inflammatory processes in several ways. First, the circulating levels of soluble RAGE are associated with various inflammatory diseases in patients (15–17) and in mouse models of experimental acute lung injury (17). Second, it is thought that sRAGE can compete with full-length cell-surface RAGE for ligand binding, preventing these ligands from binding to their receptors (including RAGE) and/or exerting effects otherwise. The exogenous administration of sRAGE reduced inflammatory responses in several animal models, including models of hepatic injury (18–20), diabetic atherosclerosis (21, 22), delayed type hypersensitivity (23, 24), type II collagen-induced arthritis (24), and experimental autoimmune encephalomyelitis (25). HMGB1 is one possible candidate to be targeted by sRAGE in these diseases, and inhibition of HMGB1 might be partially responsible for the observed effects of sRAGE. Many RAGE ligands are promiscuous with regard to receptor use. The assumption that sRAGE not only prevents HMGB1 and other RAGE ligands from binding to RAGE but also prevents them from binding to other receptors is supported by the finding that administration of sRAGE also decreased the delayed-type hypersensitivity response in RAGE-deficient mice (23). In

addition, sRAGE might be involved in inflammation because it has proinflammatory and chemo-tactic properties itself via interaction with the β 2-integrin Mac-1 (26).

RAGE and HMGB1 have been implicated as mediators of lethality in abdominal sepsis caused by cecal ligation and puncture (CLP) (23, 27–29). The CLP model is less suitable to study the impact of an intervention on bacterial growth and dissemination because of the polymicrobial nature of the infection and the fact that the antibacterial response is dependent on the extent of necrosis of the cecum and the formation of a local abscess (30). Our laboratory has used a model of abdominal sepsis induced by intraperitoneal injection of *E. coli* to investigate host defense mechanisms against this most common pathogen in peritonitis (31–34). This model is suitable to study factors contributing to local growth within the peritoneal cavity and subsequent dissemination of bacteria, as well as the occurrence of systemic inflammation and organ injury. We used this model to determine the effect of sRAGE and anti-HMGB1 on host defense against septic peritonitis.

MATERIALS AND METHODS

Mice

Eight- to 10-wk-old female C57Bl/6 mice were purchased from Harlan Sprague Dawley (Horst, the Netherlands). The Institutional Animal Care and Use Committee of the Academic Medical Center, University of Amsterdam, approved all experiments.

Soluble RAGE

Murine sRAGE was prepared and purified from a baculovirus expression system as previously described (21). Any detectable lipopolysaccharide was removed by Detoxi-gel columns (Pierce Chemical, Rockford, IL) (35). sRAGE was administered intraperitoneally at a dose of 100 μ g in 200 μ L 0.5 hrs after infection, and control mice received equal volumes of vehicle (phosphate-buffered saline). In addition, a separate experiment was performed using mice treated with either sRAGE or vehicle without being infected with *E. coli*.

Anti-HMGB1 Antibodies

Polyclonal antibodies against HMGB1 box B were raised in rabbits as described previously (27) and titers were determined by immunoblotting. Anti-HMGB1 box B antibodies were affinity-purified by using cyanogens bromide-activated Sepharose beads following standard procedures. Neutralizing activity of anti-HMGB1 was confirmed in HMGB1-stimulated macrophage cultures by assay of tumor necrosis factor (TNF)- α release. In the presence of the antibody, neutralizing antibody was defined as inhibition (>80%) of HMGB1-induced TNF- α release. Anti-HMGB1 antibodies were administered intraperitoneally at a dose of 500 μ g 0.5 hrs before infection. Control mice received equal volumes of nonimmune rabbit immunoglobulin G (IgG; item I5006; Sigma-Aldrich, St. Louis, MO).

Induction of Peritonitis

Abdominal sepsis was induced by intraperitoneal injection of *E. coli* O18:K1 (10^4 colony-forming units [CFUs]) as described (32–34). Sample harvesting and processing and

determinations of bacterial loads and cell counts were performed as described (32–34). Healthy, uninfected mice were used for baseline levels ($t = 0$ hrs).

Assays

sRAGE levels were measured by enzyme-linked immunosorbent assay (R&D, Minneapolis, MN) as described (36, 37). Keratinocyte-derived chemokine (KC) and macrophage inflammatory protein 2 (MIP-2) were measured by enzyme-linked immunosorbent assay according to the instructions of the manufacturer (R&D Systems, Abingdon, UK). TNF- α , interleukin (IL)-6, monocyte chemoattractant protein (MCP)-1, and IL-10 were measured by cytometric bead array multiplex assay (BD Biosciences, San Jose, CA) in accordance with the manufacturer's recommendations. Aspartate aminotransferase and alanine aminotransferase were determined with commercially available kits (Sigma-Aldrich, St. Louis, MO) using a Hittachi analyzer (Boehringer Mannheim, Mannheim, Germany). Myeloperoxidase was measured by enzyme-linked immunosorbent assay (Hycult Biotechnology BV, Uden, the Netherlands). HMGB1 was measured by Western immunoblotting (27, 38).

Histologic Examination

Lungs and livers were harvested after 20 hrs, fixed in 4% formaldehyde, embedded in paraffin, and cut in 4- μ m-thick sections for staining procedures. Hematoxylin-eosin procedures were performed as described (39) and analyzed by a pathologist who had no knowledge of the treatment of the mice. The following parameters were scored: parenchymal inflammation, necrosis and fibrin for the livers and interstitial inflammation, pleuritis, edema, and thrombi for the lungs. Each parameter was graded on a scale of 0 to 4 (0, absent; 1, mild; 2, moderate; 3, severe; and 4, very severe). The total histology score was expressed as the sum of the score for all parameters. Neutrophil and fibrin(ogen) stainings were performed as described (40 – 43).

Statistical Analysis

Data are expressed as mean values $\pm$ SEM. Differences between groups were analyzed by Mann-Whitney U test. Values of $p < .05$ were considered to represent a statistically significant difference.

RESULTS

sRAGE Facilitates Bacterial Dissemination to Distant Organs During *E. coli* Peritonitis

To obtain insight on the effect of sRAGE on bacterial outgrowth and dissemination, mice were inoculated intraperitoneally with *E. coli* together with either sRAGE or vehicle. We established the number of *E. coli* CFUs in peritoneal lavage fluid (PLF; the primary site of infection), blood, liver, and lungs (to evaluate to which extent the infection became systemic) at 20 hrs after infection. Mice treated with sRAGE had similar CFU counts in PLF and blood as did mice treated with vehicle (Fig. 1A and B; $4.0 \times 10^7 \pm 1.1 \times 10^7$ vs. $1.02 \times 10^8 \pm 4.0 \times 10^7$, $p = .1457$ and $2.10 \times 10^8 \pm 6.8 \times 10^7$ vs. $3.87 \times 10^8 \pm 1.22 \times 10^8$ CFUs/mL, $p = .2379$, respectively). However, livers from sRAGE-treated mice clearly contained more bacteria compared with mice treated with vehicle (Fig. 1C; $2 \times 10^5 \pm 6 \times 10^4$ vs. $2.6 \times 10^7 \pm$

8×10^6 CFUs/mL, $p < .0001$). In addition, there was more bacterial outgrowth in the lungs from mice administered with sRAGE (Fig. 1D; $2.9 \times 10^7 \pm 9 \times 10^6$ vs. $1.12 \times 10^8 \pm 4.0 \times 10^7$ CFUs/mL, $p = .0117$). Therefore, administration of sRAGE was associated with an increased bacterial dissemination during *E. coli* peritonitis, predominantly to the liver. In accordance with the literature (36), circulating sRAGE levels in healthy, uninfected control mice ($t = 0$ hrs) were not detectable. sRAGE or vehicle treatment in either uninfected or *E. coli* peritonitis mice did not enhance plasma or PLF concentrations of sRAGE (all levels were below the detection limit of 62.5 pg/mL, data not shown).

Inflammatory Cell Influx and Chemokine Levels in PLF Are Not Influenced by sRAGE

The recruitment of leukocytes to the site of an infection is an essential part of the host defense to invading bacteria. sRAGE has been shown to interact with Mac-1, thereby acting as an important chemotactic stimulus for neutrophils (26). Hence, we determined leukocyte counts and chemokine levels in PLF at 20 hrs after *E. coli* injection in sRAGE-treated and vehicle-treated mice and in healthy, uninfected control mice ($t = 0$ hrs). First, we found that sRAGE treatment in uninfected mice (i.e., mice that did not receive *E. coli*) did not influence total leukocyte numbers or neutrophil influx compared to vehicle treatment (Table 1). *E. coli* injection resulted in a profound increase in total leukocyte numbers in PLF compared to 0 hrs, which was mainly attributable to neutrophil influx (Table 1). Administration of sRAGE during *E. coli* peritonitis did not result in a change in peritoneal leukocyte counts or differentials compared to administration of vehicle (Table 1). The mouse CXC chemokines KC and MIP-2 have been implicated to play an important role in the attraction of neutrophils during inflammation (44, 45). Therefore, we measured the levels of these chemokines in PLF. sRAGE administration in uninfected mice did not induce elevated KC or MIP-2 concentrations after 20 hrs (Table 1). In line with leukocyte counts and differentials in PLF in mice with *E. coli* peritonitis, concentrations of KC and MIP-2 were similar in the sRAGE-treated and vehicle-treated mice (Table 1).

Cytokine Response During *E. coli* Sepsis in PLF and Plasma Is Only Minimally Affected by Administration of sRAGE

To determine whether sRAGE influences the local or systemic release of cytokines during septic peritonitis, TNF- α , IL-6, MCP-1, and IL-10 concentrations were measured in PLF and plasma of sRAGE-treated and vehicle-treated mice 20 hrs after *E. coli* injection and of healthy, uninfected control mice ($t = 0$ hrs; Table 2). In uninfected mice, sRAGE treatment did not alter cytokine levels in PLF or plasma compared to vehicle (Table 2). Levels of MCP-1 in PLF and IL-10 in plasma were significantly higher in sRAGE-treated mice (Table 2), whereas IL-6 concentrations tended to be higher in PLF and plasma, although these differences did not reach statistical significance (Table 2).

sRAGE Treated Mice Have More Liver Damage in *E. coli*-Associated Sepsis

This experimental model of abdominal sepsis is associated with profound liver injury (32–34). Considering the strongly increased bacterial outgrowth in the livers from sRAGE-treated mice, we examined the influence of sRAGE administration on liver damage after *E. coli* injection. First, we found that on histopathological examination, sRAGE administration

in mice without *E. coli* peritonitis does not induce liver damage or inflammation at 20 hrs (Fig. 2C; “uninfected”). During abdominal *E. coli* sepsis, both sRAGE-treated and vehicle-treated mice showed mild inflammation of liver tissue, as characterized by the influx of leukocytes into the hepatic parenchyma (Fig. 2A and 2B). Although total histology scores did not differ between sRAGE-treated and vehicle-treated septic mice (Fig. 2C; 11.0 ± 1.4 vs. 6.6 ± 1.9 ; $p = .0927$), clinical chemistry showed more profound hepatocellular injury in sRAGE-treated mice. The mice that received sRAGE had higher plasma levels of aspartate aminotransferase, although alanine aminotransferase concentrations were similar compared to vehicle-treated mice (Fig. 2D; 4108 ± 474.0 vs. 2643 ± 569.1 U/L, $p = .0205$ and 2070 ± 328.8 vs. 1832 ± 335.0 U/L, $p = .6334$, respectively). The mice administered with sRAGE demonstrated more fibrin/thrombus formation (Fig. 2B, arrow and Fig. 2G; 6.5 ± 1.2 [sRAGE] vs. 3.0 ± 1.2 [vehicle], $p = .0343$) and showed markedly more fibrin(ogen) depositions (Fig. 2F vs. 2E).

sRAGE Administration in *E. coli*-Induced Septic Mice Increases Neutrophil Recruitment in the Liver Interstitium

Having shown that sRAGE facilitates bacterial dissemination to the liver, we then asked ourselves what factors might be involved in the early spread of *E. coli*. Given that leukocyte recruitment to the site of infection is an important part of host defense during bacterial infection, we next investigated influx of neutrophils in the livers. Therefore, we performed granulocyte stainings of liver tissue. Compared to vehicle-treated mice, sRAGE-administered mice showed more hepatic neutrophil influx (Fig. 3B vs. 3A). sRAGE-treated mice had higher myeloperoxidase levels (reflecting the neutrophil content of an organ) in liver homogenates (Fig. 3C; 3547 ± 396.6 [sRAGE] vs. 1993 ± 231.4 ng/mL [vehicle]; $p = .0062$). CXC chemokines have been implicated in the attraction of neutrophils to the site of an infection (44, 45). To investigate whether a difference in local chemokine levels could have influenced the neutrophil influx in the interstitial liver tissue, we determined KC and MIP-2 levels in liver homogenates. Mice that had received sRAGE showed higher levels of MIP-2 in their liver homogenates than did mice that received vehicle (Fig. 3E; 10664 ± 661.2 [sRAGE] vs. 6652 ± 589.4 pg/mL [vehicle]; $p = .0009$). KC levels were similar between the two mouse groups (Fig. 3D; 31116 ± 1206 [sRAGE] vs. 28223 ± 1351 pg/mL [vehicle]; $p = .2031$).

Hepatic Cytokine Responses Are Increased in sRAGE-Treated *E. coli*-Induced Septic Mice

Finally, we measured the proinflammatory cytokines TNF- α , IL-6, and MCP-1 and the anti-inflammatory cytokine IL-10 in liver homogenates. Levels of these cytokines were not influenced by sRAGE administration in mice without *E. coli* infection (Fig. 4A–D). During *E. coli* sepsis, TNF- α , IL-6, and MCP-1 liver concentrations were clearly elevated in the mice that received sRAGE compared to mice that received vehicle (Fig. 4A–C; 72.5 ± 11.8 vs. 16.3 ± 2.8 , $p = .0002$ [TNF- α]; 4977.0 ± 877.7 vs. 1525 ± 261.0 , $p = .0003$ [IL-6]; and 8103.0 ± 561.8 vs. 2596.0 ± 322.4 pg/mL, $p < .0001$ [MCP-1]). IL-10 levels in infected mice were higher in the sRAGE-treated mice, also (Fig. 4D; 119.7 ± 17.2 vs. 72.9 ± 4.7 pg/mL; $p = .0266$ [IL-10]).

sRAGE Injection Does Not Influence Pulmonary Inflammation During *E. coli* Sepsis

To determine the role of sRAGE in the development of inflammation in another organ even more susceptible to inflammation-induced injury, lungs were harvested at 20 hrs after *E. coli* infection. First, we assessed that sRAGE administration in the absence of *E. coli* infection (“uninfected”) does not alter lung inflammation compared to vehicle injection after 20 hrs (Fig. 5C; $.75 \pm .25$ vs. $1.3 \pm .25$; $p = .3429$). Pulmonary inflammation during *E. coli* peritonitis was present as reflected by the accumulation of leukocytes in the interstitium (Fig. 5A and 5B). During *E. coli* abdominal sepsis, the total histologic scores did not differ between sRAGE and vehicle groups (Fig. 5C; $5.0 \pm .6$ vs. $3.8 \pm .3$; $p = .1011$). Myeloperoxidase concentrations in lung homogenates, however, were slightly higher in the *E. coli*-infected mice treated with sRAGE (Fig. 5D; 7869 ± 505.4 vs. 6736 ± 258.3 ng/mL; $p = .0266$). Neutrophil stainings of lung tissue were similar in both *E. coli*-infected groups (Fig. 5E and 5F).

Elevated HMGB1 Levels in *E. coli*-Induced Abdominal Sepsis and Anti-HMGB1 Treatment Induces Elevated Bacterial Loads in Liver and Lungs

Having established that sRAGE treatment adversely affects the host response to *E. coli* peritonitis, we next questioned whether HMGB1, one of the high-affinity binding ligands for RAGE (9, 10), plays a role herein. To investigate whether HMGB1 is released in our model of abdominal sepsis, we measured HMGB1 concentrations in PLF from healthy mice and from mice 20 hrs after *E. coli* injection. HMGB1 was either not or barely measurable in PLF from uninfected mice and was clearly increased after induction of *E. coli* peritonitis (Fig. 6A; 42.5 ± 12.2 vs. $1.13 \pm .5$ ng/mL; $p < .0001$). We next asked ourselves what the contribution of endogenous HMGB1 is in host defense during peritonitis. Therefore, we treated mice with either anti-HMGB1 IgG or control IgG antibodies and quantified the number of bacteria in various body compartments 20 hrs after intraperitoneal injection of *E. coli*. Anti-HMGB1 IgG treatment resulted in an enhanced dissemination of *E. coli* from the primary site of infection, as reflected by higher bacterial loads in liver, lungs, and blood, albeit in blood the difference with vehicle-treated mice did not reach statistical significance (Fig. 6C–6E; $2 \times 10^6 \pm 6 \times 10^5$ vs. $4 \times 10^5 \pm 2 \times 10^5$; $p = .0172$ [liver]; $7.0 \times 10^7 \pm 4.1 \times 10^7$ vs. $6 \times 10^6 \pm 3 \times 10^5$, $p = .0279$ [lungs]; and $4.45 \times 10^8 \pm 2.30 \times 10^8$ vs. $3 \times 10^6 \pm 2 \times 10^6$ CFUs/mL, $p = .6038$ [blood]). In PLF, bacterial loads were virtually identical in anti-HMGB1 IgG and control IgG-treated mice (Fig. 6B; $6.3 \times 10^7 \pm 3.0 \times 10^7$ vs. $5.7 \times 10^7 \pm 4.7 \times 10^7$; $p = .9682$). Anti-HMGB1 IgG did not influence inflammatory responses (cell influx or CXC chemokine levels in PLF, cytokine release in PLF and plasma, plasma aspartate aminotransferase and alanine aminotransferase, and liver and lung pathology; data not shown).

DISCUSSION

The clinical syndrome of sepsis is the result of a systemic response of the host to a severe infection, characterized by the concurrent activation of various mediator systems. In this study we show that administration of sRAGE resulted in an enhanced bacterial outgrowth in the distant organs, liver and lungs, together with increased inflammation and damage in the liver. Additionally, we report that the administration of an antibody against one of the high-

affinity targets of sRAGE, HMGB1, led to a similar pattern in bacterial dissemination, but without enhancement of hepatocellular injury. Together these data suggest that during severe peritonitis RAGE ligands and particularly HMGB1 play a role in limiting the dissemination of *E. coli* from the primary site of infection.

Knowledge of the role of RAGE ligands in host defense during bacterial infection is limited. sRAGE-treated mice showed an unaltered mortality after induction of polymicrobial sepsis induced by CLP (23), and anti-HMGB1 administration was associated with a survival advantage even when administered 24 hrs after CLP (27, 28). In these studies, all mice received broad-spectrum antibiotics and bacterial loads were not reported. Together with the fact that host defense against CLP at least in part relies on the extent of intestinal necrosis and the formation of a local abscess (30), the possible role of RAGE ligands cannot be determined from these earlier investigations. Although our model does not resemble clinical abdominal sepsis as closely as CLP, it is a relevant tool to study the role of mediators/receptors in limiting the growth and dissemination of bacteria after a primary intra-abdominal infection and to determine the contribution of these proteins to specific immune responses (32, 42). As such, we provide evidence using sRAGE and anti-HMGB1 IgG that RAGE ligands, among which HMGB1, contribute to an effective antibacterial response. These data do not necessarily contradict the previously protective effects of anti-HMGB1 treatment with regard to CLP-induced mortality (27, 28). The immune response to bacterial infection can act as a double-edged sword, protecting the host against invading pathogens and also potentially damaging cells and tissues. It is conceivable that bacterial growth and dissemination do not impact significantly on the outcome of CLP-induced sepsis, particularly in the context of antibiotic therapy. Future research is warranted to investigate RAGE ligand-mediated antibacterial activity against other pathogens associated with abdominal sepsis without the use of antibiotics. It should be noted that the mortality curves after infection of previously healthy mice with this *E. coli* strain are very steep. Whereas low doses do not cause lethality, doses that do cause lethality almost invariably do so. The bacterial dose used here is associated with 100% lethality, with the first mice dying soon after the 20-hr time point (42). Hence, we consider this model less suitable to determine the impact on mortality and rather make use of it to study host defense mechanisms.

Peritonitis is characterized by recruitment of leukocytes to the site of infection (31, 42, 46). Theoretically, sRAGE can influence cell influx by binding HMGB1, which we show here to be released into the peritoneal cavity during *E. coli* peritonitis, thereby abrogating the chemotactic properties of HMGB1 (47). In accordance with this possibility is our recent observation in RAGE-deficient mice in which we found a reduced neutrophil influx into PLF on intraperitoneal administration of recombinant HMGB1 (48). However, we did not find a decreased neutrophil influx to the peritoneal cavity in mice treated with sRAGE. Furthermore, the concentrations of both CXC chemokines KC and MIP-2 that play an important role in the attraction of neutrophils were similar in PLF. Notably, the number of neutrophils in liver and lungs, as determined by measurement of myeloperoxidase concentrations, was higher in the sRAGE-treated mice. This was most likely the consequence of the increased bacterial load providing a more potent proinflammatory stimulus.

sRAGE-treated mice displayed more fibrin/thrombi formation in their livers, in combination with higher cytokine and increased plasma aspartate aminotransferase concentrations. This is remarkable because administration of sRAGE has been shown to diminish hepatic damage in mice subjected to ischemia and reperfusion, massive hepatectomy, and acetaminophen-induced hepatotoxicity (18–20). In addition, anti-HMGB1 IgG did not impact on liver damage, whereas anti-HMGB1 IgG decreased inflammation and liver damage after CLP (28) and attenuated hepatic ischemia/reperfusion injury (49). Considering that anti-HMGB1 IgG had less of a negative impact on the hepatic bacterial load than sRAGE, these data suggest that the detrimental effect of sRAGE on the growth of *E. coli* in the liver is only partially mediated by inhibition of HMGB1. Furthermore, it remains to be established whether sRAGE and anti-HMGB1 IgG can exert liver protective effects during abdominal sepsis. Such possible liver-protective effects might have been overruled in our peritonitis model by the increased number of bacteria in the livers of sRAGE and anti-HMGB1 IgG-treated mice, whereby sRAGE treatment was associated with enhanced hepatocellular injury because of the 100-fold higher bacterial load.

The fact that, in contrast to sRAGE, anti-HMGB1 IgG did not impact on inflammatory responses during *E. coli* sepsis suggests that RAGE ligands other than HMGB1 play a role in the host responses that were exaggerated by sRAGE. We chose to administer sRAGE 0.5 hrs after *E. coli* injection, 19.5 hrs before the mice were euthanized, because in earlier studies of acute inflammation sRAGE administration exerted effects within this time frame (19). Of note, the timing of sRAGE administration likely influences its impact on the innate immune response. As such, the effect of sRAGE described here may have been different when the injection had been delayed.

We recently reported that intraperitoneal injection of recombinant HMGB1 induces local release of cytokines and chemokines as well as influx of neutrophils in PLF by a mechanism that partially depends on RAGE and Toll-like receptor 4 (48). Our current data using anti-HMGB1 in mice infected with *E. coli* do not contradict these earlier findings. Endogenous HMGB1 could exert antibacterial effects *in vivo* either directly (50) or indirectly via amplifying the inflammatory response, thereby explaining the higher bacterial loads in the anti-HMGB1–treated mice. The unaltered proinflammatory response in the anti-HMGB1–injected *E. coli*-infected mice could be the net result of the inhibitory effect of anti-HMGB1 treatment on the proinflammatory response and also the proinflammatory effects attributable to the higher bacterial loads.

CONCLUSIONS

Peritonitis is a common cause of sepsis in humans. Intraperitoneal administration of live *E. coli* results in a clinical condition commonly associated with septic peritonitis, with diaphragmatic lymphatic drainage, systemic bacteremia, and endotoxemia. We used this model to investigate the function of endogenous RAGE ligands and, more specifically, HMGB1 in host defense against septic peritonitis. Both inhibition of multiple RAGE ligands, by the administration of sRAGE, and inhibition of HMGB1 resulted in an enhanced dissemination of *E. coli* from the primary site of infection. These results identify a novel

beneficial role for endogenous RAGE ligands, including HMGB1, in the innate antibacterial response to abdominal sepsis.

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Figure 1.

Soluble receptor for advanced glycation end product (sRAGE)-treated mice demonstrate an enhanced dissemination. Number of *Escherichia coli* colony-forming units (CFUs) in peritoneal lavage fluid (PLF) (A), blood (B), liver (C), and lungs (D) at 20 hrs after intraperitoneal injection of 5×10^4 CFUs of *Escherichia coli* in mice treated with either vehicle (white bars) or sRAGE (black bars) (n = 8–10 mice/group). Data are mean $\pm$ SEM; * $p < .05$ and *** $p < .005$ vs. vehicle-treated mice.

Figure 2.

Soluble receptor for advanced glycation end product (*sRAGE*) worsens *Escherichia coli* (*E. coli*) sepsis-associated liver damage. Mice were treated with either vehicle or *sRAGE* intraperitoneally at 0.5 hrs after 5×10^4 colony-forming units *E. coli* injection.

Representative hematoxylin-eosin stainings of liver tissue at 20 hrs after *E. coli* infection in vehicle-(A) and *sRAGE*-treated (B) mice. Original magnification $\times 10$, scale bars are shown in yellow. Arrow points out thrombus. Graphical representation of the degree of liver inflammation (C) and of fibrin and thrombi (G) determined according to the scoring system described in the Materials and Methods section. Control mice received vehicle or *sRAGE* in the absence of *E. coli* infection (“uninfected”) and “0 hrs” indicates baseline levels (C).

Plasma aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) levels (D). Representative fibrin(ogen) immunostaining of liver tissue of mice administered with either vehicle (E) or *sRAGE* (F) after injection with *E. coli*. Original magnification $\times 10$, scale bars are shown in yellow. White bars represent vehicle-treated and black bars indicate *sRAGE*-treated mice (n = 8–10 mice/group). Data are mean $\pm$ SEM; * $p < .05$ vs. vehicle-treated mice.

Figure 3. Soluble receptor for advanced glycation end product (*sRAGE*) increases hepatic neutrophil influx during *Escherichia coli* sepsis. Mice were treated with either vehicle or *sRAGE* intraperitoneally at 0.5 hrs after 5×10^4 colony-forming units *Escherichia coli* injection. Representative leukocyte antigen-6 stainings of liver tissue at 20 hrs after *Escherichia coli* infection in vehicle-(A) and *sRAGE*-treated (B) mice. Original magnification $\times 10$, scale bars are shown in yellow. Myeloperoxidase (MPO; C), keratinocyte-derived chemokine (KC; D), and macrophage inflammatory protein 2 (MIP-2; E) levels in liver homogenate in mice treated with control (white bars) or *sRAGE* (black bars) ($n = 8-10$ mice/group). Data are mean $\pm$ SEM; ** $p < .01$ vs. vehicle-treated mice, *** $p < .005$ vs. vehicle-treated mice.

Figure 4.

Treatment of soluble receptor for advanced glycation end product (sRAGE) elevates cytokine concentrations in the liver in *Escherichia coli* (*E. coli*)-induced septic mice. Mice were treated with either vehicle or sRAGE intraperitoneally at 0.5 hrs after 5×10^4 colony-forming units *E. coli* injection. Control mice received either vehicle or sRAGE without *E. coli* infection ("uninfected") and "0 hrs" indicates baseline levels. Tumor necrosis factor (TNF)- α (A), interleukin (IL)-6 (B), monocyte chemoattractant protein (MCP)-1 (C), and IL-10 (D) levels of liver homogenates in mice treated with vehicle (white bars) or sRAGE (black bars) (n = 8–10 mice/group). Data are mean $\pm$ SEM; *p < .05 vs. vehicle-treated mice, ***p < .001 vs. vehicle-treated mice.

Figure 5.

Influence of soluble receptor for advanced glycation end product (sRAGE) on pulmonary inflammation. Mice were treated with either vehicle or sRAGE intraperitoneally at 0.5 hrs after 5×10^4 colony-forming units *Escherichia coli* (*E. coli*) injection. Representative hematoxylin-eosin stainings of lung tissue at 20 hrs after *E. coli* infection in vehicle- (A) and sRAGE-treated (B) mice. Original magnification $\times 20$, scale bars are shown in yellow. Graphical representation of the degree of lung inflammation (C) determined according to the scoring system described in the Materials and Methods section and myeloperoxidase (MPO) levels in lung homogenate (D) in mice treated with vehicle (white bars) or sRAGE (black bars) ($n = 8-10$ mice/group). Control mice received vehicle or sRAGE in the absence of *E. coli* infection ("uninfected") and "0 hrs" indicates baseline levels (C). Representative leukocyte antigen-6 stainings of lung tissue at 20 hrs after *E. coli* infection in vehicle- (E) and sRAGE-treated (F) mice. Original magnification $\times 10$, scale bars are shown in yellow. Data are mean $\pm$ SEM; * $p < .05$ vs. vehicle treated-mice.

Figure 6.

Effects of treatment of anti-high-mobility group box 1 (*HMGB1*) immunoglobulin G (*IgG*) during *Escherichia coli* (*E. coli*)-induced sepsis. Wild-type mice were inoculated intraperitoneally with 5×10^4 colony-forming units (*CFUs*) of *E. coli* and euthanized after 20 hrs. Local (peritoneal lavage fluid [*PLF*]; *A*) *HMGB1* levels. Bacterial loads in *PLF* (*B*), blood (*C*), liver (*D*), and lungs (*E*) were determined in wild-type mice treated with anti-*HMGB1* *IgG* or control *IgG*. Data are mean $\pm$ SEM ($n = 9$ – 10 mice/group); * $p < .05$ vs. control-treated mice.

Table 1

Leukocyte counts and chemokine levels in peritoneal fluid

	Uninfected		<i>Escherichia coli</i> Peritonitis	
	t = 0 hrs		t = 20 hrs	
	Vehicle	sRAGE	Vehicle	sRAGE
Cell count, $\times 10^4/\text{mL}$ PLF				
Total	54.4 $\pm$ 3.5	60.9 $\pm$ 2.5	62.0 $\pm$ 7.1	215.4 $\pm$ 23.2
Neutrophils	0.0 $\pm$ 0.0	1.8 $\pm$ 0.4	4.1 $\pm$ 1.1	189.4 $\pm$ 19.2
Macrophages	50.9 $\pm$ 4.1	56.9 $\pm$ 1.7	53.0 $\pm$ 8.1	33.6 $\pm$ 6.1
Chemokines, pg/mL, PLF				
KC	<DL	<DL	<DL	24180 $\pm$ 3172
MIP-2	<DL	<DL	<DL	3587 $\pm$ 957
				4495 $\pm$ 876

<DL, less than detection limit. sRAGE, soluble receptor for advanced glycation end products; PLF, peritoneal lavage fluid; KC, keratinocyte-derived chemokine, MIP-2, macrophage inflammatory protein-2.

Escherichia coli peritonitis indicates mice that received 5×10^4 colony-forming units *Escherichia coli* intraperitoneally in contrast to uninfected mice that did not receive *Escherichia coli* at t = 0 hrs. Mice were euthanized at time points as indicated. Levels of healthy, uninfected control mice (t = 0 hrs) are shown to depict baseline levels.

Table 2

Cytokine levels

	Uninfected		<i>Escherichia coli</i> Peritonitis	
	t = 20 hrs		t = 20 hrs	
	t = 0 hrs		Vehicle	sRAGE
Cytokines, pg/mL				
TNF- α				
PLF	<DL	<DL	63.3 $\pm$ 7.0	65.5 $\pm$ 5.3
Plasma	7.4 $\pm$ 2.1	9.4 $\pm$ 5.0	354.5 $\pm$ 80.6	337.2 $\pm$ 86.8
IL-6				
PLF	<DL	<DL	707.5 $\pm$ 180.0	1570 $\pm$ 345.8; $p = .05$
Plasma	<DL	2.6 $\pm$ 2.6	14538 $\pm$ 3542	25667 $\pm$ 4308; $p = .08$
Monocyte chemoattractant protein-1				
PLF	<DL	<DL	2614 $\pm$ 375.4	7209 $\pm$ 540.8 ^b
Plasma	<DL	20.0 $\pm$ 3.8	5054 $\pm$ 852.5	5024 $\pm$ 701.5
IL-10				
PLF	<DL	4.8 $\pm$ 3.2	154.7 $\pm$ 45.7	237.2 $\pm$ 42.2
Plasma	<DL	1.0 $\pm$ 1.0	466.1 $\pm$ 98.0	1093 $\pm$ 161.3 ^a

sRAGE, soluble receptor for advanced glycation end products; TNF- α , tumor necrosis factor- α ; interleukin, IL; PLF, peritoneal lavage fluid; <DL, less than detection limit.^a $p < .005$ vs. vehicle-treated *Escherichia coli* mice.^b $p < .0001$ vs. vehicle-treated *Escherichia coli* mice. *Escherichia coli* peritonitis indicates that mice received 5×10^4 colony-forming units *Escherichia coli* intraperitoneally in contrast to uninfected mice that did not receive *Escherichia coli* at t = 0 hrs. Mice were euthanized at time points as indicated. Levels of healthy, uninfected control mice (t = 0 hrs) are shown to depict baseline levels.